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Award Number: DAMD17-98-1-8578

TITLE: Development of a Transgenic Stem Cell Model of Prostate Cancer

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REPORT DATE: September 1999

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

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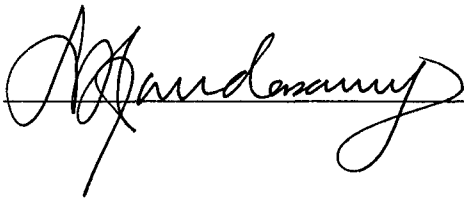
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<b>1. AGENCY USE ONLY (Leave blank)</b>		<b>2. REPORT DATE</b> September 1999	<b>3. REPORT TYPE AND DATES COVERED</b> Annual (1 Sep 98 - 31 Aug 99)	
<b>4. TITLE AND SUBTITLE</b> Development of a Transgenic Stem Cell Model of Prostate Cancer			<b>5. FUNDING NUMBERS</b> DAMD17-98-1-8578	
<b>6. AUTHOR(S)</b> Robert E. Reiter, M.D.				
<b>7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)</b> University of California Los Angeles, California 90095-1406  <b>E-MAIL:</b> rreiter@mednet.ucla.edu			<b>8. PERFORMING ORGANIZATION REPORT NUMBER</b>	
<b>9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)</b>  U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012			<b>10. SPONSORING / MONITORING AGENCY REPORT NUMBER</b>	
<b>11. SUPPLEMENTARY NOTES</b>  This report contains colored photos				
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<b>13. ABSTRACT (Maximum 200 Words)</b>  Purpose: The overall goal of our proposal is to develop a new transgenic model of prostate cancer by targeting transformation to prostatic basal cells using the prostate stem cell antigen promoter (PSCA). Major Findings: A 9kb region upstream of the PSCA coding sequence was isolated and demonstrated to drive prostat-specific expression in vitro. This region was subsequently tested in vivo using a GFP reporter construct. Analysis of the PSCA promoter-GFP transgenic mice have demonstrated relatively prostate-specific expression of GFP. GFP expression in these mice is regulated developmentally, with prominent expression localized to the ductal tips of the prostate. Promoter activity appears to be androgen responsive and androgen independent. Conclusion: The PSCA promoter can drive target gene expression to the prostate of mice. Expression is restricted to a subset of prostatic cells, potentially the progenitor cells of the prostate. We are currently using this promoter to create new transgenic models of prostate cancer.				
<b>14. SUBJECT TERMS</b> Prostate Cancer				<b>15. NUMBER OF PAGES</b>  12
				<b>16. PRICE CODE</b>
<b>17. SECURITY CLASSIFICATION OF REPORT</b> Unclassified	<b>18. SECURITY CLASSIFICATION OF THIS PAGE</b> Unclassified	<b>19. SECURITY CLASSIFICATION OF ABSTRACT</b> Unclassified	<b>20. LIMITATION OF ABSTRACT</b>  Limited	

NSN 7540-01-280-5500

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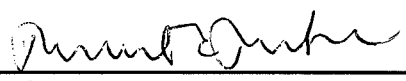
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## INTRODUCTION

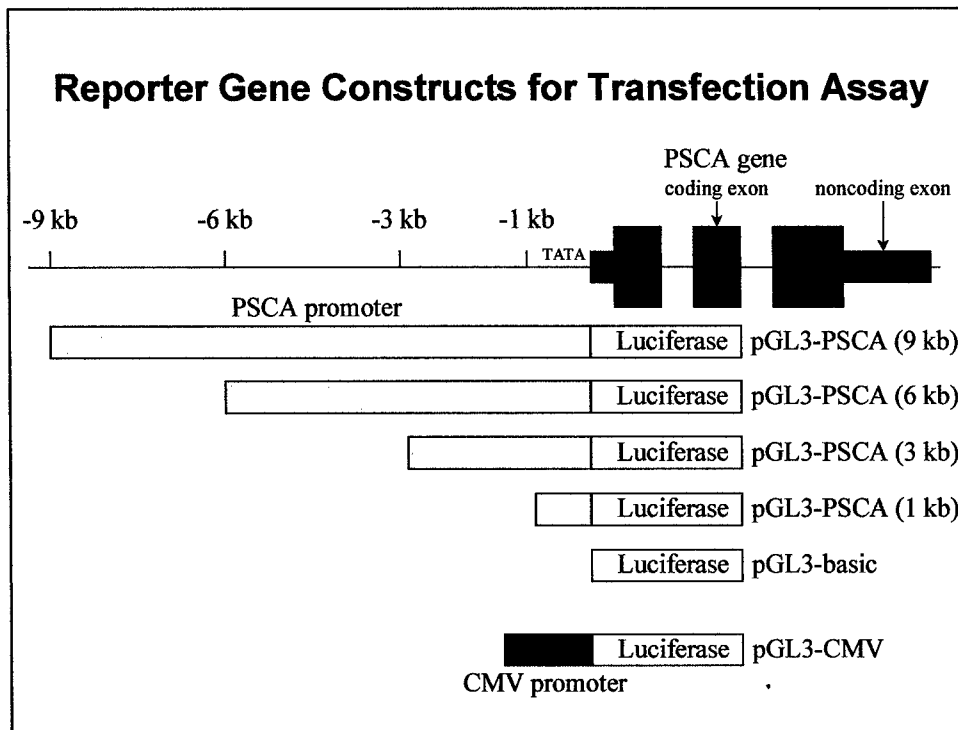
The overall goal of this proposal is to create a new transgenic model of prostate cancer by targeted transformation of basal cells using the Prostate Stem Cell Antigen (PSCA) promoter. The proposal is based on the major hypothesis that prostate cancer arises from malignant transformation of a subset of prostatic basal cells. PSCA is a cell surface antigen whose expression in normal tissues is largely restricted to prostatic basal cells. The specific Aims of our proposal therefore are to 1) clone the PSCA promoter region and identify the sequences capable of driving prostate expression (years 1-2) and 2) to create a model of prostate cancer by targeting SV40 T antigen expression to PSCA-responsive prostatic cells (years 2-3). We have already completed a majority of the work outlined in Aim 1 and have begun the experiments planned in Aim 2.

## PROGRESS REPORT

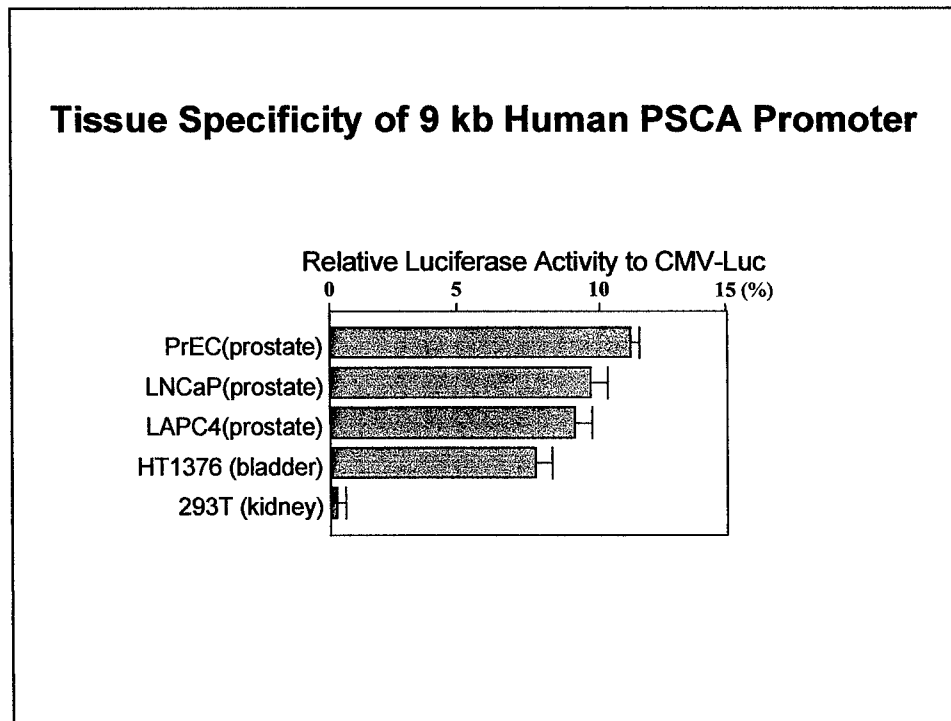
### **Specific Aim 1: Delineation of PSCA genomic sequences capable of driving transgene expression in the prostate.**

Task 1. Cloning and characterization of mouse and human PSCA genes. The human and mouse PSCA genes were isolated by probing genomic libraries with the respective cDNA clones. 9 and 14kb lambda clones were obtained for the murine and human genes, respectively. These clones were also used to obtain ~120kb BAC clones, in case the lambda clones did not contain all the sequences necessary for prostatic expression. The three known exons of the human and murine PSCA genes were mapped within the lambda and BAC clones and the regions 5' to exon 1 were sequenced. Although other PSCA family members (i.e. Thy-1/Ly-6) contain 4 exons, we have only been able to identify 3 exons in murine and human prostate. The existence of only 3 exons is further supported by the finding of a putative TATA box immediately upstream of the first exon in both the murine and human clones. A potential androgen response element was also identified by sequence analysis of this upstream region. We concluded that the region upstream of exon 1 likely contains the PSCA promoter and enhancer.

Fragments ranging from 1-9kb 5' to exon 1 were isolated by restriction digestion of the lambda and BAC clones. These were cloned upstream of a luciferase reporter gene in order to identify putative promoter/enhancer regions for PSCA (Figure 1). These constructs were transiently transfected into prostatic and nonprostatic cell lines in order to test for activity and prostate specificity. As shown in Figure 2, the 9kb construct was active in the prostatic cell lines LNCaP and LAPC-4, but not in the kidney cell line 293T. Equivalent results were obtained with the 6 kb construct. We also saw activity in a bladder cancer cell line, which is consistent with the recent finding in our laboratory that PSCA is expressed at low levels in normal transitional epithelium, as well as in a subset of bladder cancers. Other nonprostatic cell lines tested were negative. These results suggested that the 6 and 9kb human genomic constructs contained the necessary elements for prostate (and bladder) specific expression.



**FIGURE 1: PSCA-luciferase constructs.**



**FIGURE 2. Activity of the 9kb PSCA promoter in prostatic and nonprostatic cell lines.**



Because we found a potential androgen response element in the PSCA promoter region, we also tested for luciferase activity in prostatic cell lines before and after the administration of testosterone. As predicted, the PSCA promoter was responsive to androgen, with a 50-100 fold increase in luciferase activity in the presence of testosterone. It is important to note, however, that the PSCA promoter is not dependent on androgen, as it was highly active in prostate epithelial cells (PreC) which do not express androgen receptor (Figure 2). Interestingly, PreC cells express a basal cell phenotype, supporting the hypothesis of this proposal that PSCA is expressed in basal cells, is androgen independent, yet is also androgen responsive.

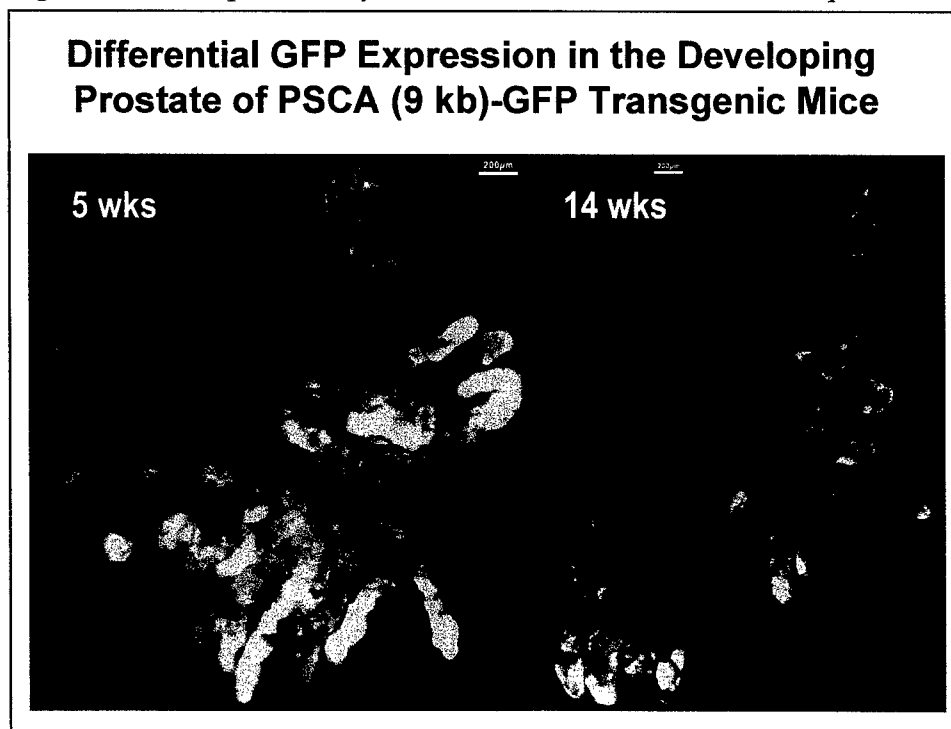
Task 2. Construction of PSCA-GFP reporter constructs. The 6 and 9kb human PSCA promoter sequences were cloned upstream of the GFP reporter gene using the Clontech pEGFP vector. The activity of this construct was tested in transient transfection assays as described above, with identical results. Stable LNCaP cell lines expressing GFP under the control of the PSCA promoter were also derived for animal experiments.

Tasks 3 and 4. Production and analysis of PSCA-GFP transgenic mice. Based on the hypothesis that the human PSCA promoter would be more tissue-specific than the murine PSCA promoter, the 9 and 6kb human PSCA-GFP constructs were microinjected into C57BL/6XC3H pronuclei to generate PSCA-GFP transgenic mice. Southern blot and PCR assays to detect the presence of the transgene were developed in parallel. Founder mice containing the transgene were bred and analyzed for GFP expression. The F1 generation mice of five DNA-positive founders were analyzed. Mice were analyzed initially by simply dissecting the prostatic lobes and nonprostatic tissues and looking at them directly under the immunofluorescent microscope. Subsequently, individual ducts were microdissected and analyzed, as well as whole mount sections of the individual organs. Two independent lines of mice were found to express GFP in their prostates. Both lines also had detectable GFP in skin. All other tissues analyzed were negative, including stomach, intestine, kidney, seminal vesicle, spleen, liver, salivary glands, brain, lung, thymus, testis and heart. The F1 generation of a single founder line expressed GFP in the bladder; however, the F2 generation lost this expression while maintaining prostatic expression, presumably because of the insertional site of the transgene. As described in the original proposal, the founder line most positive for GFP was maintained and evaluated extensively as follows.

a) Developmental regulation of GFP. GFP expression was studied pre and postpubertally. In 5 day old mice, GFP expression was located in the proximal ducts of the expanding prostate, but not in the distal tips. At 3-6 weeks of age (around the age of puberty), GFP expression was concentrated primarily in the distal tips of the ducts, but extended into the proximal ducts as well (Figure 3). After puberty, at age 14 weeks, all GFP activity was focused in the distal tips, with virtually no expression seen proximally (Figure 3). The significance of these findings is currently being studied. The existing literature on prostatic development in mice indicates that most of the proliferative activity occurs in the distal tips, suggesting that the PSCA promoter may identify regions of relatively high mitotic activity. However, there is little proliferative activity at 14 weeks and a great deal of activity at 5 days (at which point there was little GFP expression in the distal tips),

suggesting that this interpretation may not be correct. Staining of ductal tips at 14 weeks with a marker of proliferative activity, Ki 67, also shows few actively dividing cells. We are currently staining ducts at various points during development for high and low weight cytokeratins, bcl-2 and Ki 67 in order to identify more accurately the cell type expressing GFP--basal stem vs. basal intermediate vs. secretory intermediate vs. secretory.

Regardless, these preliminary results demonstrate that the PSCA promoter can target GFP



expression fairly

**FIGURE 3. GFP expression at 5 weeks and 14 weeks. Expression in 5week old mice extends from the distal tips proximally, whereas expression at 14 weeks in the distal tips only.**

specifically to a subset of cells in the prostatic epithelium. These results are distinct from transgenic mice developed using the probasin promoter, in which the transgenes are expressed more ubiquitously in the prostatic epithelium.

b) Hormonal regulation of PSCA-GFP in transgenic mice. Based on our transient transfection data, we predicted that GFP expression would be androgen independent and responsive. Preliminary castration experiments of 14 week old PSCA-GFP transgenic mice confirm that GFP expression decreases after androgen ablation. Studies are ongoing to determine the level of basal PSCA expression after castration and the cell type in which it is expressed. Similar studies are also being performed at different points in development.

**Specific Aim 2. Use of the SV40 T antigen under the control of the PSCA promoter to create a novel transgenic model of prostate cancer.** Our overall hypothesis is that PSCA is a marker of the cell which is transformed during prostate cancer initiation. Aim

1 identified the PSCA promoter elements necessary to direct transgene expression in the prostate. Additional work is necessary to clarify the specific cell type in which GFP is expressed. We are also performing RNA in situ hybridization studies of normal prostate with antisense PSCA to confirm that GFP expression mimics endogenous PSCA expression. Based on the preliminary result that GFP is expressed under the control of the current PSCA construct, we have proceeded to make PSCA-SV40 T antigen constructs, have worked out the details for screening founders, and have injected pronuclei. The first eight founder mice are being evaluated at present.

### **KEY RESEARCH ACCOMPLISHMENTS:**

1. Cloning of murine and human PSCA genomic sequences.
2. Identification of PSCA promoter/enhancer sequences
3. In vitro demonstration of promoter activity and tissue specificity.
4. Construction of PSCA-GFP transgene.
5. Derivation of PSCA-GFP transgenic mice.
6. Analysis of PSCA-GFP transgenic mice.

### **REPORTABLE OUTCOMES:**

1. Abstract-Gordon Research Conference, see attached
2. Application for NIH Program Project Grant (R Reiter and O Witte, Co-PI's of project #4, Charles Sawyers, overall PI) for construction of new animal models for prostate cancer.

### **CONCLUSIONS:**

The overall goal of our proposal was to isolate the PSCA promoter and use it as the basis for creation of a new transgenic model of prostate cancer. To date we have isolated the promoter, characterized it in vitro and used it to drive expression of a reporter gene in transgenic mice. These results are extremely promising in that we achieved relatively prostate-specific expression of the transgene in the prostate. More importantly, the transgene is detected in a subset of prostatic cells, consistent with our hypothesis that PSCA would direct expression to a subset of prostate basal or progenitor cells. Future work is geared toward analysis of the PSCA-GFP mice and creation of a prostate cancer model based on T antigen expression in PSCA-regulated cells.

*Abstract: Gordon Research Conference, August, 1999*

**The 9 kb human PSCA promoter directs reporter gene expression to the ductal tips of prostate in transgenic mice.**

Tetsuro Watabe<sup>1</sup>, Mark Lin<sup>2</sup>, Annemarie Donjacour<sup>3</sup>, Gerald R. Cunha<sup>3</sup>, Owen N. Witte<sup>1</sup> and Robert E. Reiter<sup>2</sup>

<sup>1</sup>Howard Hughes Medical Institute, <sup>2</sup>Department of Urology, University of California, Los Angeles, <sup>3</sup>Department of Anatomy, University of California, San Francisco

One of the important questions in prostate cancer research is whether prostate cancer arises from malignant transformation of differentiated secretory cells or pluripotent basal cells of prostate epithelium. The ability to answer this question has been hindered by the lack of basal cell specific promoter sequences which could be used for targeted transformation of basal cells. Human Prostate Stem Cell Antigen (PSCA) is expressed predominantly in prostate, and its transcripts are restricted to basal stem/progenitor cells in normal prostate epithelium. In order to make an alternative transgenic mouse model of prostate cancer that originates from basal cells, we examined the human PSCA promoter in *in vitro* tissue culture and in an *in vivo* transgenic mouse system. A 14 kb genomic fragment encoding the human PSCA gene was isolated and characterized. A 9 kb fragment containing 5' flanking sequences was subcloned into a luciferase reporter gene construct. Transient transfection of various human cells with the reporter construct suggested that the 9 kb region of the PSCA promoter has tissue specificity for prostate and bladder. Transfection of PrEC cells, primary cultured cells that represent human prostatic epithelial cells, with various deletion constructs suggested that the cis-elements that are necessary to induce PSCA expression may reside between -3 kb and -6 kb in the PSCA promoter. In order to further analyze the temporal and spatial regulation of the PSCA promoter, we generated transgenic mouse lines carrying the 9 kb PSCA promoter and GFP (green fluorescent protein) cDNA. One of three independent lines of mice showed GFP expression specifically in prostate and skin. Interestingly, the GFP expression in 8 weeks old prostate was restricted to ductal tips. This is the region of the prostate that has the highest proliferative activity. Ductal tips can give rise to prostate ductal structures when recombined with urogenital sinus mesenchyme and grafted under the renal capsule, indicating that stem cells may be present. However, this ability to regenerate prostatic tissue is not restricted to the distal regions in which the transgene is expressed. In immature males, (3 weeks old) GFP expression was more widely spread to the proximal ducts. These results suggest that the 9 kb PSCA promoter may serve as a good tool to generate a new transgenic mouse model of prostate cancer.



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